

European Mink *Mustela lutreola* L., 1761

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European mink *Mustela lutreola* L., 1761 is considered one of the most endangered mammalian species in the world, due to its ongoing population depletion, both in terms of the actual number of individuals and area occupied. The species was originally spread over most of continental Europe, but nowadays only three wild, isolated, declining populations occupying less than 3% of the former range survive with only about 5000 individuals are estimated persisted in the wild. The alarming situation of the species is proven by its categorization as critically endangered (CR) by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, and it is listed in Annex II to the Bern Convention on the Conservation of European Wildlife and Natural Habitats, Annexes II and IV (priority species) of the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, and in The Carpathian List of Endangered Species (critically endangered species (CR)). Despite this, studies in the field of genetics of *M. lutreola* are limited and urgently need to be completed, especially in the context of the progressing extinction process and the disappearance of its numerous populations in France, Belarus, and Russia, among others. The rapidly shrinking and vanishing genetic resources will largely never be studied and described, which is an irreversible loss from cognitive and practical points of view. The meagre data on interpopulation genetic diversity may significantly impair the efficacy of the implemented activities for restitution of the European mink, especially in the context of conservation breeding and species reintroduction. Notably, only (conservation) genetics can provide tools to rescue species affected by the extinction vortex, which, in turn, requires more research initiatives in the conservation genetics of the European mink.

Keywords: critically endangered species ; conservation genetics ; conservation biology ; captive breeding ; conservation breeding ; European Mink Centre ; European Mink Day ; Polish Society for Conservation Genetics LUTREOLA ; European mink ; *Mustela lutreola*

1. Introduction

Due to ongoing population depletion, both in terms of the actual number of individuals and area occupied, European mink *Mustela lutreola* L., 1761 is considered one of the most endangered mammalian species in the world ^{[1][2]}. The species was originally spread over most of continental Europe, but nowadays only three wild, isolated, declining populations occupying less than 3% of the former range survive ^[3]. About 5000 individuals are estimated to persist in the wild ^[3]. Reintroduced populations were established in Estonia and Germany ^[2]. The main cause of the situation of this species is habitat loss and fragmentation, overhunting, and the effects of introduced invasive American mink *Neovison vison* ^[4]. The alarming situation of the species is proven by its categorization as critically endangered (CR) by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, and it is listed in Annex II to the Bern Convention on the Conservation of European Wildlife and Natural Habitats, Annexes II and IV (priority species) of the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, and in The Carpathian List of Endangered Species (critically endangered species (CR)) ^{[2][5]}.

Despite this, the number of studies on European mink, reflected in the number of scientific papers devoted to the species, is relatively low. The digital repository of the National Centre for Biotechnology Information (NCBI) records less than 60 scientific articles devoted to various aspects of the biology of the species. In comparison, the same repository lists as many as 2000 articles related to the giant panda *Ailuropoda melanoleuca* ^[6]. Studies in the field of genetics of *M. lutreola* are limited and urgently need to be completed, especially in the context of the progressing extinction process and the disappearance of its numerous populations in France, Belarus, and Russia, among others ^[2]. Among the scientific articles devoted to the species and recorded in the NCBI repository, only ~15 concern genetic issues (not including multispecies phylogenetic analyses not directly focused on European mink) ^[6]. The rapidly shrinking and vanishing genetic resources will largely never be studied and described, which is an irreversible loss from cognitive and practical points of view ^{[7][8]}. The meagre data on interpopulation genetic diversity may significantly impair the efficacy of the implemented activities for restitution of the European mink, especially in the context of conservation breeding and species reintroduction ^{[7][9][10][11][12]}.

Notably, only (conservation) genetics can provide tools to rescue species affected by the extinction vortex ^[13], which, in turn, requires more research initiatives in the conservation genetics of the European mink ^{[14][15]}.

The pioneer genetic studies on European mink concerning cytogenetics were conducted in the former USSR. To date, works by Volobuev and Ternovsky ^[16], Volobuev et al. ^[17], Graphodatsky et al. ^[18], Graphodatsky et al. ^[19], and Graphodatsky and Radjabli ^[20], strongly affiliated with the Siberian Branch of the Russian Academy of Sciences in Novosibirsk (Russia), are the primary sources of information on *M. lutreola* karyotype. Further research on the genetics of European mink relates primarily to genetic markers ^{[9][21]}, the phylogenetic relationships of the species ^{[22][23][24][25][26]}, noninvasive methods of identification ^[27], assessment of intraspecies genetic diversity ^{[8][14][28][29][30]}, and mitochondrial DNA (mtDNA) studies ^{[24][28][31][32]}. Worth mentioning are studies concerning molecular ecology on issues relating to European mink and implementing genetic research methods ^{[33][34][35][36]}.

2. Genetic Research on European Mink and Conservation Issues

The overall conclusion from the analysis of the history and scope of genetic research on European mink is a gradual increase in the genomic representativeness of the nucleotide sequences used. This trend is in line with the widespread transition from genetics to genomics, which is also (and perhaps especially) observed in conservation genetics ^{[37][38][39]}. Important in the case of *M. lutreola*, advanced genomics (whole-genome and reduced-representation approaches) can help to describe and explain adaptive genetic variations, resolving phylogenetic and phylogeographic questions, identifying adaptive alleles, as well as identifying and quantifying inbreeding, hybridization and introgression in a more accurate way than genetics ^{[40][41][42][43][44]}. Whole-genome sequencing also provides valuable knowledge about the origin and evolutionary history of endangered species and defines units for conservation, thus helping to improve conservation strategies ^{[42][43]}. For a nonmodel endangered species, such as European mink, obtaining a reference genome is essential ^{[45][46]}.

Genomic methods considerably improved conservation efforts toward some mammalian endangered species. The whole-genome approach was previously applied to the conservation of African wild dog *Lycaon pictus* to detect inbreeding and population-specific selection ^[47]. Genomics provides tools for population evaluation monitoring and management of small populations in the wild and in captivity, species delineation, and to enhance wildlife health management and identify risk factors for genetic disorders of endangered primates ^{[48][49][50]}. Conservation genomics helped to identify the conservation implications of admixture in the Eastern wolf *Canis lupus lycaon* ^[42]. Wright et al. ^[51] identified several candidate genes that may be associated with variation in the breeding success of the Tasmanian devil *Sarcophilus harrisii*. From the *M. lutreola* population genetics perspective, the same research demonstrated that individual heterozygosity was not associated with breeding success in captivity but was negatively associated with litter sizes of breeding females ^[51].

The correct selection of an appropriate sample size for population genetics research seems to be a neglected issue. Determination of the optimal level of sampling effort required for adequate characterization of the intraspecies genetic variation is of fundamental importance ^[52]. Too many samples than required for accurate estimation of genetic diversity increases costs and workload and lengthens the analysis, whereas too small a sample size results in significant errors in estimating the genetic diversity ^{[53][54][55]}. Particular sensitivity of mtDNA and microsatellite markers to sample sizes in addressing questions related to interpopulation genetic diversity, phylogenetics, and phylogeography leads to recommendations of using genomic data over microsatellites or a limited number of mitochondrial or/and nuclear single nucleotide polymorphism markers for genetic studies ^{[52][56][57][58]}. The limited availability of samples for genomic studies on *M. lutreola* also needs to be further investigated, as sampling size may be a significant limitation of genomics in conservation ^[43].

In future research on the genetic diversity of the surviving European mink populations, much more attention should be paid to optimization of the sample size, as sampling many individuals per population but using a small number of genetic markers does not contribute to accurate and reliable results ^{[55][59]}. Genomics offers a solution: Genome-wide techniques, such as restriction site-associated DNA sequencing (RAD-seq), to acquire a large number of single nucleotide polymorphisms (SNPs), allowing finer identification of population structure and stronger determination of patterns of isolation-by-distance than with microsatellites, with a smaller sample size ^{[41][60]}. The need to address the small number of available samples is crucial given the declining wild populations of European mink. Obtaining genetic samples from the rediscovered, relic wild populations of the species in the Dniester Delta in Ukraine is also important as a completely recently rediscovered Caucasian population or a presumptive Carpathian population ^{[2][61][62]}. The application of eDNA-based species detection is promising in rediscovery research projects.

Based on the analysis of the research work completed to-date in the field of *M. lutreola* genetics, the following research issues remaining to be addressed and resolved are as follows:

Research Issues Per Se:

1. Establishing a karyotype reference standard;
2. Initiation and completion of the whole-genome sequencing project (an improved scaffolded genome of *M. putorius* GenBank assembly accession GCA_902207235.1, and a platinum quality genome available for *M. erminea*, RefSeq assembly accession GCF_009829155.1, could be used for reference-based assembly or designing primers for any genomic location for targeted sequencing);
3. Development of a genome-scale (mitogenome-scale) SNPs panel, optimal for the study of inter- and intrapopulation genetic diversity, and the species phylogeny (possible revision of taxonomic status at the genus level) and phylogeography.

Conservation Issues:

1. Resolving the issue of undertaking conservation actions (including translocations) of wild persisting populations (scientifically-informed decisions regarding whether to treat them as a single or separate management units, which is especially relevant given the plans for the inclusion of the Spanish breeding program in the EEP program and plans for obtaining new founders from the wild Romanian population ^{[12][41]});
2. Assessment of the impact of the breeding process on the development of traits essential for survival in the wild (adaptation to captivity) and the role of (re)introduced individuals in shaping the gene pools of wild populations (potential outbreeding and loss of unique adaptations, which could be assessed using captive-breeding experiments, in which individuals from distinct populations are hybridized to check if a loss of fitness is occurring);
3. Phylogeographic reconstruction in terms of origin of the French–Spanish population.

Promotional and Organizational Issues:

1. Encouraging the scientific community to undertake research related to European mink genetics, as well as its promotion and popularization (e.g., in the context of scientific social responsibility (SSR));
2. Scientific cooperation and networking (including sharing experiences, sharing samples, mentorship, and transfer of research results to conservation practice);
3. Development of protocols for the preservation and biobanking of the species' genetic resources, which could be based on the extensive achievements and experience in this field with the Black-footed ferret ^{[12][63][64]}.

Maran et al. ^[12] listed the molecular genetic studies linked to the long-term management plan for European mink aiming to (1) determine if the population introduced onto Hiiumaa Island (Estonia) is in need of genetic supplementation in the coming years, (2) study the genetic diversity of the founders originating from the Western European population and the wild Eastern European population to determine whether this follows the same pattern found in earlier studies, and (3) encourage interest in the genome sequencing of European mink.

The question of the potential influence of reduced genetic diversity (e.g., in terms of MHC ^[65]) on the viability and survival of the wild and reintroduced populations also remains unresolved. Research initiatives should be undertaken to help resolve how genome alteration in each demographic influences *M. lutreola* species viability in a global change scenario ^{[66][41]}.

Just as important as experimental research are reviews and (meta-)analytical works that indicate how gained genetic knowledge can be used to solve specific problems facing the conservation of *M. lutreola*, thereby indicating the application value of this knowledge.

The initiative to establish the European Mink Centre (www.europeanminkcentre.org) is worth mentioning; an internet platform, acting as a digital repository, information hub, and think tank dedicated to conservation biology, including conservation genetics, of the species ^[67]. The Centre's aim is to stimulate and facilitate research in the field of *M. lutreola* conservation genetics ^[67]. The promotion of the achievements of conservation genetics is of particular importance in the European mink case, as the success of the implemented conservation measures depends to a large extent on the favor of decision-makers and the general public. Hence, initiatives such as the European Mink Day proposed in 2015 by the Polish Society for Conservation Genetics LUTREOLA on 31 March may have an indirect and positive impact on creating research interest in *M. lutreola* and implementation of genetic research results in its conservation ^[68].

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